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Effects of different agricultural systems on organic matter and aggregation of a medium-textured soil in subtropical region

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ABSTRACT

This work evaluates the impact of different agricultural systems on the humic fractions of organic matter (SOM) and soil aggregation in a medium-textured soil in a subtropical region of Brazil. Three managed areas and a reference area were evaluated: permanent pasture (PP), no-till (NT), no-till+Brachiaria (NT+B) and native forest (NF). Deformed soil samples were collected in the layers 0-0.05; 0.05-0.10; 0.10-0.20 and 0.20-0.40 m, and unformed soil samples in the layers 0-0.05 and 0.05-0.10 m. After collection, total organic carbon (TOC) analysis, chemical fractioning of SOM and aggregate stability analysis were performed. The PP area presented the highest TOC and C-FA contents in the first three layers evaluated. In the 0.10-0.20 m layer, the C-HUM contents in the NT+B area were 1.9 times higher than in the NT area, and in 0.20-0.40 m they were 6.6 times higher. The PP area obtained the best structural indicators, especially higher WMD, GMD and % of macroaggregates. The two areas using direct seeding presented a percentage of macroaggregates higher than 37.28% in the superficial layer. The PP management provided increases in TOC levels and, consequently, humic substances, besides the formation of more stable aggregates. The NT+B system showed promise in increasing TOC and humic substances, especially at depth, even with a short implementation period. The increase in TOC and humic substances provided by the grasses contributes to the formation of more stable aggregates and, consequently, to the improvement of the quality of medium-textured agricultural soils.

Keywords: aggregate stability, carbon fractions, climate change, humic substances.

Efeitos de diferentes sistemas agrícolas na matéria orgânica e na agregação de um solo de textura média em região subtropical

RESUMO

O presente trabalho teve como objetivo avaliar o impacto de diferentes sistemas agrícolas sobre as frações húmicas da matéria orgânica (MOS) e a agregação do solo, sob solo de textura média em uma região subtropical do Brasil. Foram avaliadas três áreas manejadas e uma área de referência: pastagem permanente (PP), plantio direto (PD), plantio direto+*Brachiaria* (PD+B) e mata nativa (NF). Foram coletadas amostras de solo deformadas nas camadas 0-0,05; 0,05-0,10; 0,10-0,20 e 0,20-0,40 m, e amostras de solo indeformadas nas camadas 0-0,05 e 0,05-0,10 m. Após a coleta, foram realizadas análises de carbono orgânico total (COT), fracionamento químico da MOS; análise de estabilidade de agregados. A área PP apresentou os maiores teores de COT e C-AF nas três primeiras camadas avaliadas. Na camada de 0,10-0,20 m os teores de C-HUM na área PD+B foram 1,9 vezes maiores que na área PD, e em 0,20-0,40 m foram 6,6 vezes maiores. A área PP obteve os melhores indicadores estruturais, especialmente maior DMP, DMG e % de macroagregados. As duas áreas com plantio direto apresentaram percentual de macroagregados superior a 37,28% na camada superficial. O manejo do PP proporcionou aumentos nos teores de COT e, consequentemente, de substâncias húmicas, além da formação de agregados mais estáveis. O sistema PD+B mostrou-se promissor no aumento do COT e das substâncias húmicas, principalmente em profundidade, mesmo com um curto período de implantação. O aumento do COT e das substâncias húmicas proporcionado pelas gramíneas contribui para a formação de agregados mais estáveis e, consequentemente, para a melhoria da qualidade dos solos agrícolas de textura média.

Palavras-chave: alterações climáticas, estabilidade de agregados, frações de carbono, substâncias húmicas.

1. INTRODUCTION

Soil quality (SQ) is fundamental to maintaining sustainable agricultural systems that meet global food, fiber, and energy needs (Damian *et al.*, 2023). In recent decades, SQ has been discussed by the scientific community worldwide (Simon *et al.*, 2022; Thomaz and Antoneli 2022; Valani *et al.*, 2022; Burak *et al.*, 2022; Pinto *et al.*, 2022) and defined as the ability of a particular soil type to function within the limits of the natural or managed ecosystem to maintain crop productivity (Valani *et al.*, 2022). Given the demand for food to feed the world's population, human activities are causing changes in natural ecosystems (Fao, 2006). Intensification of land management through tillage can have negative consequences for soil biogeochemical cycles and lead to degradation if not adequately managed (Santos *et al.*, 2022).

Therefore, systems are constantly being investigated to look for strategies to mitigate the negative effects. Some studies indicate that pastures have a high potential for sequestering carbon (C), as estimates show that C stocks may be 50% higher in pastures than in forests worldwide (Fao, 2006; Damian *et al.*, 2023). Well-managed pastures not only help to increase C stocks, but also contribute to the formation of stable aggregates (Rosset *et al.*, 2019; Ozório *et al.*, 2019); this greater stabilization favors the infiltration of water into the soil, reducing compaction, reducing the risk of erosion (Damian *et al.*, 2023).

In contrast to well-managed pastures, production systems with no-till soybean and maize cultivation (NT), where there is no crop rotation, can slowly contribute to improving edaphic quality in terms of increasing C stocks, especially in subtropical and tropical regions where climatic factors hinder the permanence of SOM in the soil (Rosset *et al.*, 2016; Ofstehage and Nehring, 2021). In addition, in several regions of Brazil, pasture is increasingly cultivated with maize as a second crop. This practice, in addition to maximizing agricultural productivity, aims to increase C content and promote improvements in soil properties (Farias *et al.*, 2022; Rosset *et al.*, 2019) and reduce fertilizer use (Nunes *et al.*, 2020) in different soil types and textures and geographic regions (Pedruzzi *et al.*, 2022; Maia *et al.*, 2019).

The impact of soil management systems can be directly studied with quantitative and qualitative analyses of SOM (Rosset *et al.*, 2016; 2022; Assunção *et al.*, 2019). The main studies evaluating the impact of these management systems use quantification of the contents and stocks of the chemical fractions of SOM (Rosset *et al.*, 2016; Collins *et al.*, 2019), since the different systems significantly affect the content of fulvic acids (FA), humic acids (HA), and humic substances (HUM) (Tadini *et al.*, 2022a; Assunção *et al.*, 2019). In addition to the studies dealing with SOM, the analysis of aggregate stability is fundamental to the understanding of SQ (Rosset *et al.*, 2019), through its direct relationship with chemical (Silva *et al.*, 2019b; Lacerda-Junior *et al.*, 2019; Luz *et al.*, 2019), physical (Falcão *et al.*, 2020; Ozório *et al.*, 2019), and biological properties (Luz *et al.*, 2019; Soares *et al.*, 2019; Castioni *et al.*, 2018).

Considering the above and the need for studies on the edaphic quality of different production systems with different implementation times, soil types and textures, especially in tropical and subtropical regions (Pedruzzi *et al.*, 2022; Maia *et al.*, 2019; Santos *et al.*, 2019), the hypothesis of this study is that the long management history in well-managed pastures, as well as the introduction of *Brachiaria* as an intercrop with maize in the second crop in only 4 years, can favor the process of humification of SOM and does not cause changes in the structural properties of the soil. Against this background, this study investigated whether pasture, no-till and no-till systems with *Brachiaria* in winter change the properties of organic matter and soil structure in medium textured soils.

2. MATERIAL AND METHODS

2.1. Location, climate and soil collections from the study areas

Soil samples were collected in farming systems with known implementation history in the municipality of Terra Roxa, western Paraná, Brazil (Figure 1). The climate of the region is subtropical (Cfa), according to the Koeppen classification (Caviglione *et al.*, 2000). According to a detailed soil survey conducted by the State of Paraná (Bhering, 2007), the area was classified as Argissolo Vermelho-Amarelo Distrófico, which has a sandy texture in the superficial horizons (Santos *et al.*, 2018). The classification corresponded to the Paleudalfs in the USDA Soil Taxonomy (Soil Survey Staff, 2014) or the Rhodic Acrisols in the FAO classification system (Iuss Working Group WRB, 2015), which have 666, 147 and 187 g kg^{-1} of sand, silt and clay, respectively, in the 0-0.40 m layer (Santos *et al.*, 2018).

Four different areas were evaluated. The trajectories are shown in Figure 2:

- Area of permanent pasture (PP) of 4.1 ha (24º11´37.77" S - 54º06´49.17" W, 307 m elevation). The area consists of pasture with Coast Cross species (*Cynodon dactylon* (L.) Pers), which is considered resistant to low temperatures and with a high leaf/stem ratio. The area has been continuously grazed by dairy cows for 44 years, with a livestock stocking rate of 2 units/animal per hectare, with periodic reforms about every 15 years and the application of 2.1 Mg ha^{-1} of limestone.
- Area with no-till (NT) of 11 ha (24º11´27.82" S - 54º06´52.04" W, 325 m altitude) tilled successively with soybeans (summer) and corn (second crop). Historically, this area consisted of native forests of the Atlantic Forest biome, which were cleared in the 1970s. It was later managed with conventional tillage in succession of soybean/corn for 20 years and no-till for the last 24 years. The area is fertilized every 3-4 years with limestone (about 1.2 Mg ha-1) or dolomitic limestone (1.2 Mg ha-1). In addition, agricultural gypsum is applied during the same period when limestone is

fertilized, which is the recommended dose due to the presence of exchangeable aluminum. Generally, 200 kg ha-1 of formulated NPK in the ratio 04-30-10 is applied on the area when soybean is sown, and 200 kg ha-1 of formulated NPK in the ratio 10-15-15 is applied when corn is sown.

- Area with no-till soybean (summer) and maize in consortium with Brachiaria ruziziensis (second crop) (NT+B) of 28 ha (24º11´31.10" S – 54º06´52.52" W, 323m elevation). Historically, this area consisted of native forest of the Atlantic Forest biome, which was cleared in the 1970s and later managed for 20 years with conventional cropland under soybean/corn succession. In the last 20 years, the area has also been managed under no-till with soybeans and corn, and in the last 4 years, *Brachiaria ruziziensis* has been introduced in an integrated crop with corn as a second crop. Calcium is applied to the area every 4 years to correct soil acidity, with an average application of about 2 Mg ha^{-1}. For soybean cultivation, an average of 200 kg ha^{-1} of NPK formulate is applied in a 15-15-15 ratio in addition to KCl in the cover. For winter corn seeding, the area is treated with about 240 kg ha^{-1} of the formulated NPK in the 15-15-15 ratio and ammonium sulfate in the cover. In addition, soybean and corn crops are inoculated prior to seeding.
- Native forest area (NF) of the Atlantic forest biome with the phyto physiognomy of a deciduous seasonal forest of 28 ha (24º11´31.10" S - 24º06´52.52" E, 307 m altitude) - NF consisting of a permanent protected area, isolated, without access of animals, without anthropic modification.

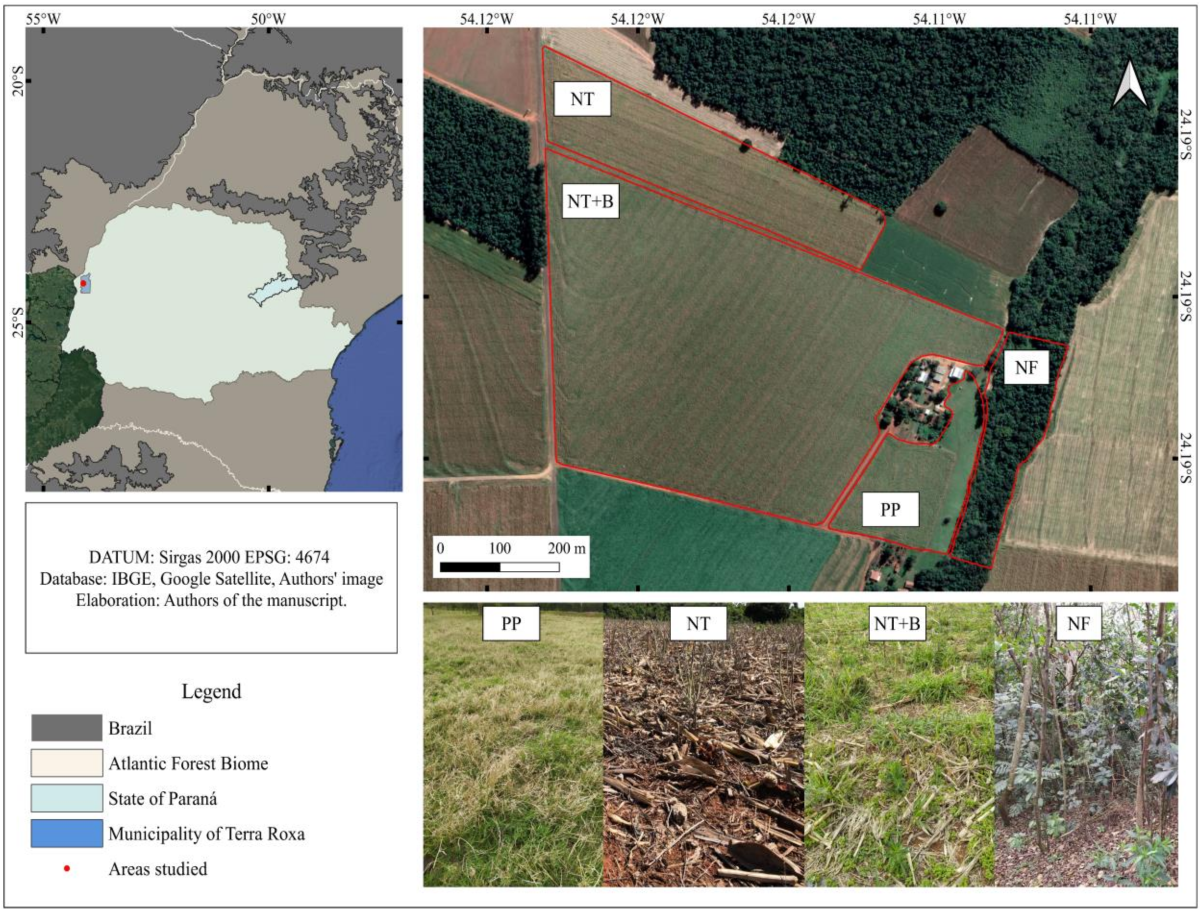

Figure 1. Experimental location map, with data from the use systems studied in the municipality of Terra Roxa, Paraná, Brazil (Source: QGIS, version 3.28 "Firenze"). PP: Permanent pasture, NT: no-till, NT+B: no-till + *Brachiaria*, NF: Native forest.

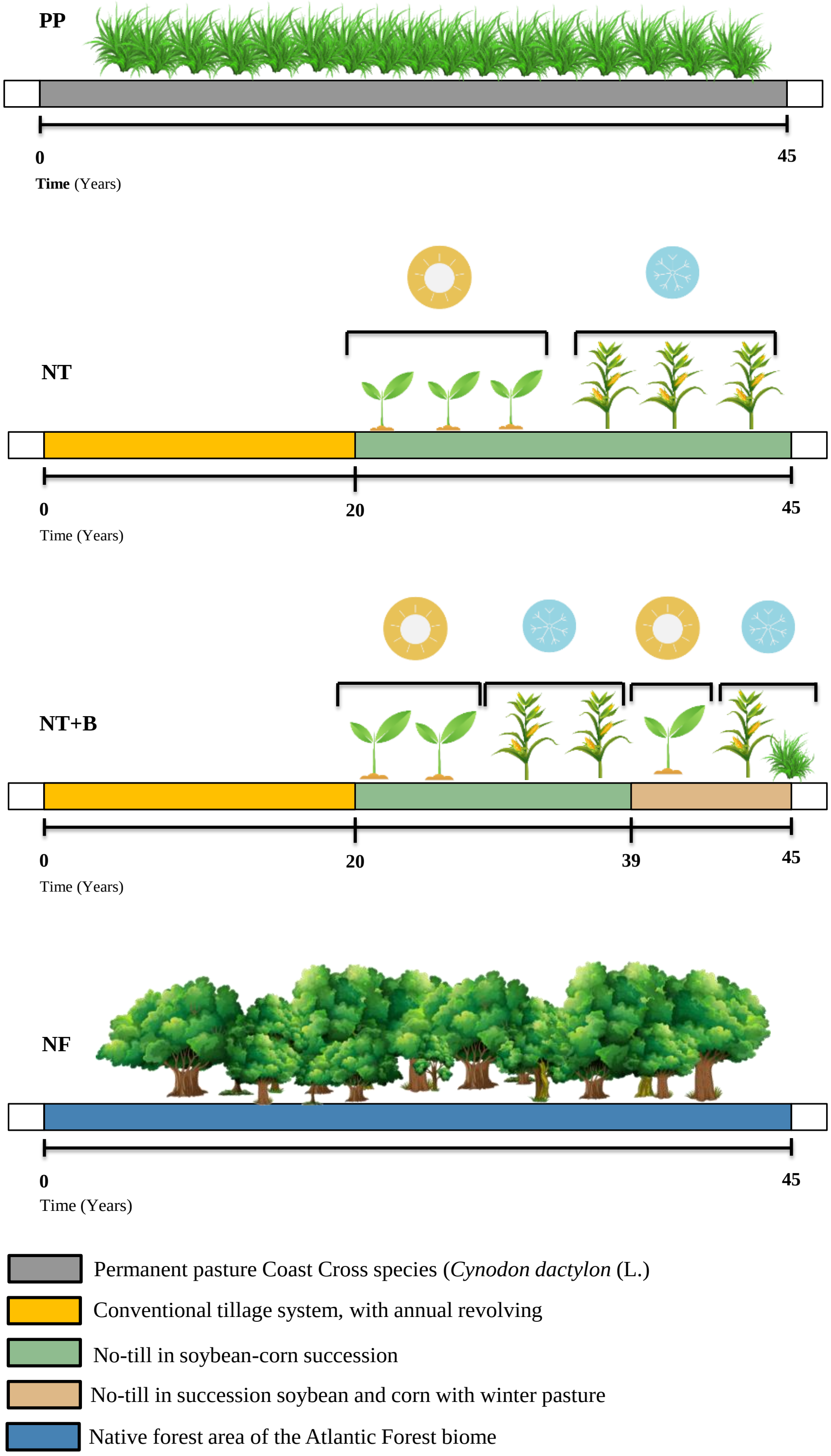

Figure 2. Soil management history of the study areas. PP: Permanent pasture, NT: No-till, NT+B: No-till + *Brachiaria*, NF: Native forest.

The samples were collected in September 2019 after the soybean harvest in the NT and NT+B areas; in the NT+B area, the *Brachiaria* had not been desiccated before collection. It is important to note that all areas are close to each other, as can be seen in Figure 1, and have the same characteristics in terms of relief, climate and soil class. For the collections, five 400 m2 plots were demarcated in each study area where soil samples were taken. Each plot represented a replicate, and each composite sample was represented by 5 simple samples within the evaluated systems in the 0-0.05; 0.05-0.10; 0.10-0.20; and 0.20-0.40 m layers. In addition, undeformed samples with a volumetric ring with a volume of 100 cm3 were taken in layers 0-0.05; 0.05-0.10; 0.10-0.20; and 0.20-0.40 m, and undeformed samples in layers 0-0.05 and 0.05-0.10 m, where the soil structure was preserved in a monolith with dimensions of 0.2 x 0.2 x 0.05 m, were taken. In order to chemically characterise the soil in the study areas, analyses were carried out in the layers 0-0.20 and 0.20-0.40 m, the results of which are listed in Table 1.

Table 1. Soil chemical characterization of the different land use systems in the 0-0.20 and 0.20-0.40 m layers.

Systems	pH	P	K^+	Ca^{2+}	Mg^{2+}	Al^{3+}	H+Al	SB	CTC	V%	m%
		mg.dm^{-3}	cmol$_c$ dm^{-3}								
	0-0.20 m										
PP	5.61	93.57	0.23	6.32	1.95	0.00	4.72	8.47	13.19	64.51	0.00
NT	6.86	36.29	0.21	4.77	1.51	0.00	0.25	6.52	6.75	96.42	0.00
NT+B	6.41	50.96	0.19	3.40	1.01	0.00	1.27	4.90	6.17	78.51	0.00
NF	4.32	14.52	0.19	2.28	0.7	0.43	6.15	3.09	9.25	32.51	14.82
	0.20-0.40 m										
PP	5.98	43.29	0.40	4.16	1.66	0.00	2.36	6.04	8.40	71.72	0.00
NT	5.87	39.36	0.21	2.54	1.18	0.19	2.15	3.87	6.02	64.46	4.98
NT+B	5.78	38.32	0.11	2.14	0.75	0.07	2.29	3.05	5.34	57.28	2.32
NF	4.05	4.11	0.08	0.59	0.70	1.14	5.47	1.32	6.79	19.41	46.21

PP: Permanent pasture, NT: No-till, NT+B: No-till + *Brachiaria*, NF: Native forest. Chemical characterization - Calcium Chloride (pH); Mehlich (P and K^+); KCl 1N (Ca^{+2}, Mg^{+2} and Al^{+3}); Calcium Acetate pH 7.0 (H + Al).

2.2. Analyses carried out

Bulk density (Db) analysis was performed according to the methodology of (Almeida *et al.*, 2017). Total organic carbon (TOC) was determined by oxidizing the MOS with potassium dichromate in a sulfuric acid medium under constant heating and titrated with an ammoniacal ferrous sulfate solution (Yeomans and Bremner, 1988). The chemical fractionation of MOS was performed according to the differential solubility technique developed by the International Society for Humic Substances (Swift, 1996), as described by Benites *et al.* (2003). The fractions (humic substances - HS) were divided into fulvic acid (FA), humic acid (HA) and humic substances (HUM) and then the carbon content (C) of each fraction was determined (Yeomans and Bremner, 1988). The following ratios were calculated from the C analyzes of HA, AH and HUM to verify the humification processes of the MOS: HA/FA and alkaline extract (AE)/HUM. In addition, SH stocks were calculated using the equivalent mass method (Reis *et al.*, 2018).

For aggregate stability analysis, the air-dried samples were first manually dissected along the aggregate lines of weakness so that the entire sample was converted into aggregates ranging in size from 8.00 mm to 4.00 mm. A 50 g portion was taken from these aggregates, which were wetted with water by capillary action for 10 minutes on filter paper. The samples were then sieved in water by the method described by Kemper and Chepil (1965) in a Yoder-type mechanical shaker (Yoder, 1936) on a set of sieves with 2.00; 1.00; 0.50; 0.25 and 0.125 mm mesh. After sieving in water, the weighted mean diameter (WMD) (Kiehl, 1979) and geometric mean diameter (GMD) (Kemper and Rosenau, 1986) were calculated using the mass obtained in each sieve class. After the WMD calculations, the degrees of order (LOrd) were determined (Vezzani, 2001), changing the original methodology by replacing the percentage of rock body mass > 2.00 mm with the WMD of the aggregates. In addition, the percentage of aggregates retained on the sieves was divided into 3 classes according to Costa Junior *et al.* (2012), which represent macroaggregates (> 2.00 mm), meso aggregates (0.250 mm - 2.00 mm) and microaggregates (< 0.250 mm).

Data analysis was based on a completely randomized experimental design. Data were tested for normality and homogeneity of variance using the Shapiro-Wilk test and Bartlett's test. Subsequently, data were subjected to analysis of variance with application of F-test and means were compared using R Core Team (2020) software with t - Student's test p=0.05. All tests were performed using the ExpDes.pt package (Ferreira, 2018b). As complementary techniques, Principal Component Analysis - PCA (Silva *et al.*, 2022) was used for multivariate analysis. Correlations between soil organic matter chemical fraction data and soil aggregation variables were evaluated using Pearson correlation, assuming p=0.05 as the significance criterion.

3. RESULTS

3.1. Chemical fractions of soil organic matter

In the permanent grassland (PP), the highest total organic carbon (TOC) contents were observed in the first three layers studied, with contents exceeding 27.80 g kg^{-1} at the surface. In the 0.20-0.40 m layer, the highest TOC contents were observed in PP and no-till + *Brachiaria* (NT+B), with contents of 11.05 and 7.05 g kg^{-1}, respectively. The no-till (PD), PD+B and native forest (FN) systems were also similar in terms of TOC content along the evaluated profile (0-0.40 m) (Table 2). In the PP area, the highest levels of fulvic acids (C-FA) were observed up to 0.20 m, with levels of 4.64, 3.44, and 2.66 g kg^{-1}, in layers 0-0.05; 0.05-0.10; and 0.10-0.20 m, respectively. In layers 0-0.05 and 0.05-0.10 m, the NT, NT+B and NF domains were similar, and in the last two layers, the NF domain had the lowest C-FA contents.

For humic acid (C-HA) contents, PP, NT and NF areas did not differ in layers 0.05-0.10; 0.10-0.20 and 0.20-0.40 m, with higher contents. In NT+B, the lowest contents were observed in layers 0-0.05; 0.10-0.20 and 0.20-0.40 m. In PP, NT+B and NF, the highest contents of humic substance (C-HUM) were observed in layers 0.10-0.20 and 0.20-0.40 m, respectively. The different land use systems did not differ in the 0.05-0.10 m layer for this most recalcitrant fraction of the SOM (Table 2).

Stocks of fulvic acids (Stock-FA), humic acids (Stock-HA), and humic substances (Stock-HUM) showed the same pattern of differences observed for the C content of each fraction. In the PP area, the highest values of Stock-FA were observed in the first three layers, with Stock values that were 85, 111, and 145% higher than in the NF area, in layers 0-0.05; 0.05-0.10; and 0.10-0.20 m, respectively. In layers 0-0.05 and 0.05-0.10 m, the NT, NT+B, and NF areas were similar for the stock-FA. The highest stock-HA was also observed at PP, especially in the layer in the 0-0.05 m stratum (Table 2).

For stock-HUM in the 0.05-0.10 m layer, the different land-use systems differed, with values ranging from 6.82 to 12.68 mg ha^{-1}. In the layers 0.10-0.20 m and 0.20-0.40 m, the areas PP, NT+B and NF had higher contents of stock-HUM. It is worth noting that in NT stock-HUM were observed, which corresponded to 51.82 and 14.98% of the total amount obtained from the surface NT+B in the layers 0.10-0.20 and 0.20-0.40 m, in other words, when the insertion of *Brachiaria* in the system, there was an increase in the percentage of intermediate carbon in terms of stability (Table 2).

In the first two layers evaluated, the areas did not differ in the ratio of humic acids to fulvic acids (C-HA/C-FA). In the 0.10-0.20 m stratum, the managed plots were conspicuous by lower values than the NF area. In the last layer evaluated, the PP and NF systems showed the highest HA/FA ratios. The managed systems showed the lowest values for the alkaline extract/C-HUM

(AE/C-HUM) ratio in the 0-0.05 m layer. Comparing the AE/C-HUM values of the NT and NT+B areas in the subsurface layer, lower AE/C-HUM values were observed in the NT+B system (Table 2).

Table 2. Total organic carbon (TOC), fulvic acid carbon (C- FA), humic acid carbon (C-HA) and humin (C-HUM), fulvic acid carbon stock (Stock-FA), humic acid carbon stock (Stock-HA) and humin (Stock-HUM), HA/FA ratio and alkali-humin extract (AE/HUM-C) of the different management systems under medium-textured soil.

MS	TOC	C-FA	C-HA	C-HUM	Stock-FA	Stock-HA	Stock-HUM	HA/FA	AE/C-HUM
	g kg^{-1}				Mg ha^{-1}				
					0-0.05 m				
PP	27.89a	4.64a	4.36a	14.29a	6.49a	6.10a	20.02a	0.94a	0.66ab
NT	17.60b	2.68b	2.73b	12.44a	3.75b	3.82b	17.42a	1.01a	0.44b
NT+B	14.38b	1.82b	1.01c	6.79b	2.55b	1.41c	9.51b	0.68a	0.41b
NF	18.24b	2.50b	1.98b	4.65b	3.50b	2.77b	6.51b	0.77a	1.58a
					0.05-0.10 m				
PP	18.72a	3.44a	2.52a	8.15a	5.36a	3.97a	12.68a	0.72a	0.74a
NT	8.93b	2.09b	1.77ab	5.04a	3.25b	2.76ab	7.85a	0.86a	1.06a
NT+B	9.94b	1.33b	0.92b	6.03a	2.07b	1.44b	9.39a	0.69a	0.38a
NF	10.87b	1.63b	1.23b	4.38a	2.54b	1.91b	6.82a	0.77a	0.77a
					0.10-0.20 m				
PP	13.89a	2.66a	1.71a	5.74a	4.47a	2.87a	9.64a	0.64b	0.81a
NT	6.35b	1.75b	1.35ab	3.12b	2.94b	2.27ab	5.25b	0.76b	1.00a
NT+B	7.71b	1.33bc	0.92b	6.03a	2.23bc	1.55b	10.13a	0.69b	0.38b
NF	7.69b	1.08c	1.79a	7.77a	1.82c	3.02a	13.04a	2.06a	0.37b
					0.20-0.40 m				
PP	11.05a	2.53a	1.18a	5.51a	4.03a	1.87a	8.76a	0.48a	0.69b
NT	5.99b	2.03ab	1.48a	1.16b	3.24ab	2.35a	1.84b	0.72b	3.67a
NT+B	7.05ab	1.23b	0.40b	7.71a	1.96b	0.64b	12.28a	0.37b	0.23b
NF	5.61b	0.36c	1.48a	7.51a	0.57c	2.36a	11.95a	6.46a	0.24b

Averages followed by equal letters in the column, in each layer, do not differ by the t-student test ($p \leq 0.05$). MS: Management systems, PP: Permanent pasture, NT: no-till, NT+B: no-till + Brachiaria, NF: Native forest.

3.2. Soil Aggregate Stability

In the PP, we observed the highest values of weighted mean diameter (WMD) and geometric mean diameter (GMD) in the 0-0.05 m (Figure 3A) and 0.05-0.10 m (Figure 3B) layers, with values exceeding 3.87 mm for WMD and 2.84 mm for GMD, respectively. The NT, NT+B and NF ranges were similar in the two layers studied, with WMD values varying between 1.46 and 3.04 mm and GMD between 0.74 and 2.02 mm.

The aggregate order level (OLev) result showed a similar pattern to the WMD and GMD results, with the highest OLev values in the PP area in both layers studied, with a value 2.48 times higher than that of the reference area in the 0-0.05 m layer and 3.65 times higher in the 0.05-0.10 m layer.

In the PP area, a higher percentage of aggregates retained in the macroaggregate class (> 2.00 mm) was observed in the two layers studied, with values higher than 80% in the 0-0.05 m layer and higher than 70% in the 0.05-0.10 m layer (Figures 4 A and B). The NT, NT+B and

NF areas did not differ, with 48.42; 37.28 and 56.29% in the 0-0.05 m layer and 21.66; 25.59 and 37.25% in the 0.05-0.10 m layer, respectively. The macroaggregate area expressed at PP was 29.04% higher than the NF area in the first layer and 37.78% higher in the 0.05-0.10 m layer.

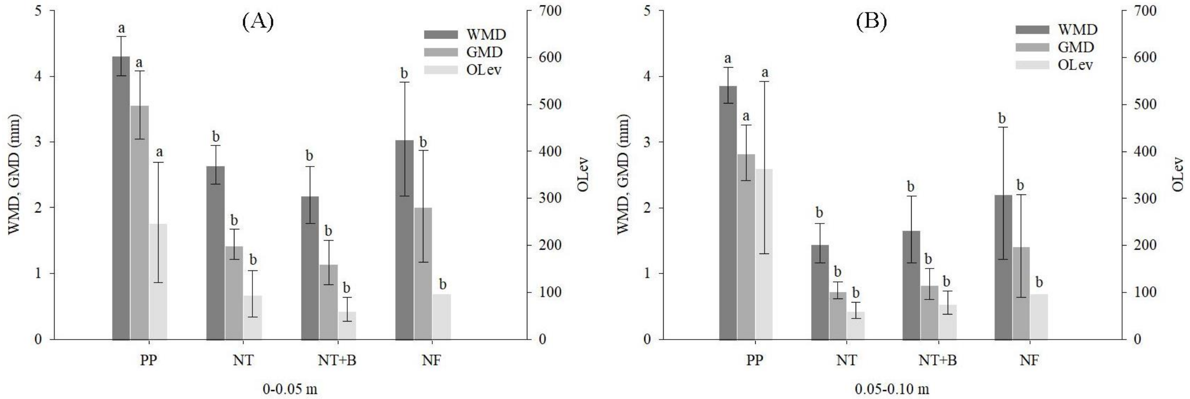

Figure 3. Weighted mean diameter (WMD), geometric mean diameter (GMD) and aggregates order level (OLev) in different management systems under medium texture soil in layers 0-0.05 (A) and 0.05-0.10 m (B). PP: Permanent pasture, NT: No-till, NT+B: No-till + *Brachiaria*, NF: Native forest.

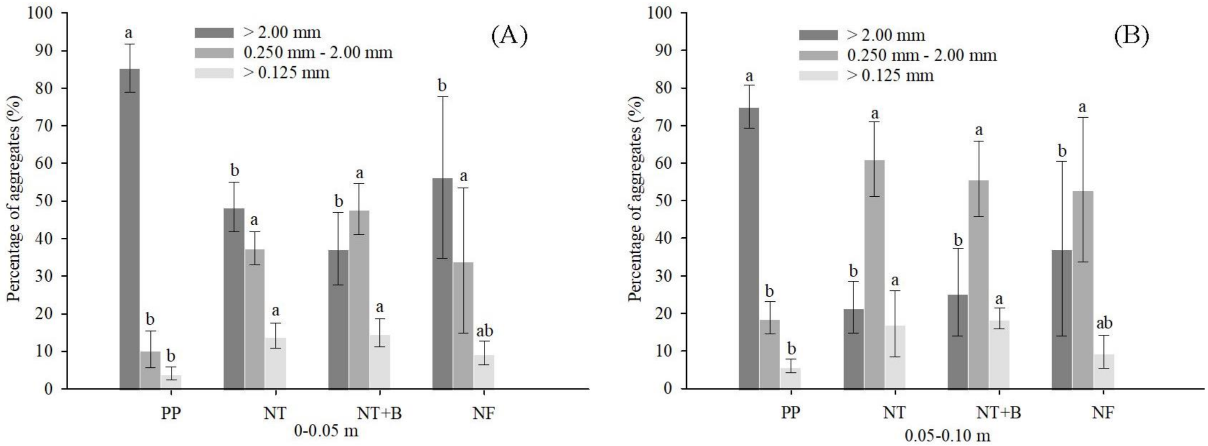

Figure 4. Percentage of aggregates of the different use systems evaluated. > 2.00 mm: macroaggregates; 0.250 mm-2.00 mm: mesoaggregates; > 0.125 mm: microaggregates. PP: Permanent pasture, NT: No-till, NT+B: No-till + *Brachiaria*, NF: Native forest in the layers 0-0.05 m (A) and 0.05-0.10 m (B).

3.3. Multivariate and correlation analysis between variables

In the principal component analysis (PCA), the first two axes explained 86.7% of the data variations, allowing us to verify the separation of the PP area from the other assessed areas (Figure 5). The variables TOC, C-FA, C-HA, C-HUM, Stock-FA, Stock-HA, Stock-HUM showed the strongest association with PP. In addition to variables related to humic substances, PP was associated with soil aggregation variables, WMP, GMD and macroaggregates. Variables representing the proportion of aggregates with smaller size (0.250 mm and 2.00 mm and < 0.250 mm) were associated with the areas NT, NT+B and NF.

A positive correlation was observed between the results of TOC, WMD and GMD and macroaggregates, with R2 greater than 0.6 for the three variables, and it was also possible to observe an inversely proportional correlation between TOC and microaggregates. In addition, the contents and stocks of C- FA, C- HA and C- HUM were correlated with WMD, GMD and macroaggregates, indicating that they influence the formation of larger aggregates (Figure 6).

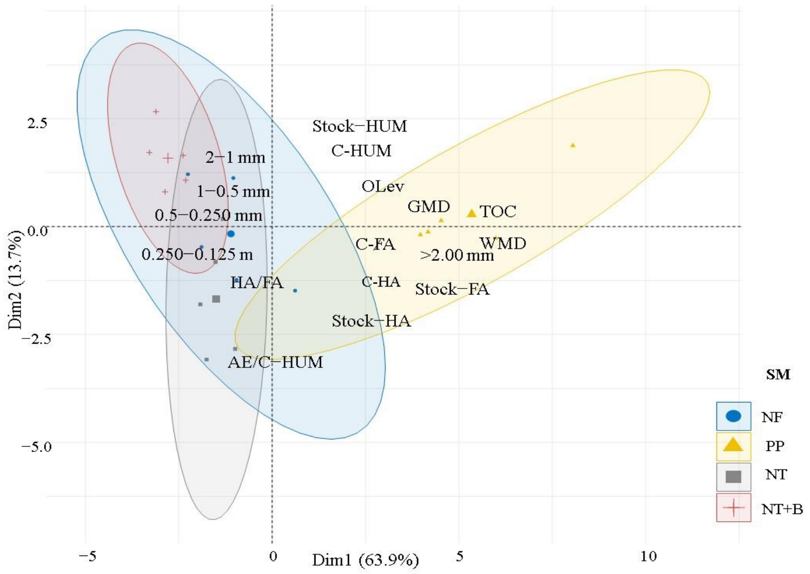

Figure 5. Principal component analysis (PCA): total organic carbon (TOC), carbon of fulvic acids (C-FA), humic acids (C-HA), and humin (C-HUM), carbon stock of fulvic acids (Stock-FA), humic acids (Stock-HA) and humine (Stock-HUM), weighted mean diameter (WMD), geometric mean diameter (GMD), macroaggregates (> 2.00 mm), mesoaggregates (0.250 mm - 2.00 mm), and microaggregates (< 0.250 mm). PP: Permanent pasture, NT: No-till, NT+B: No-till + *Brachiaria*, NF: Native forest.

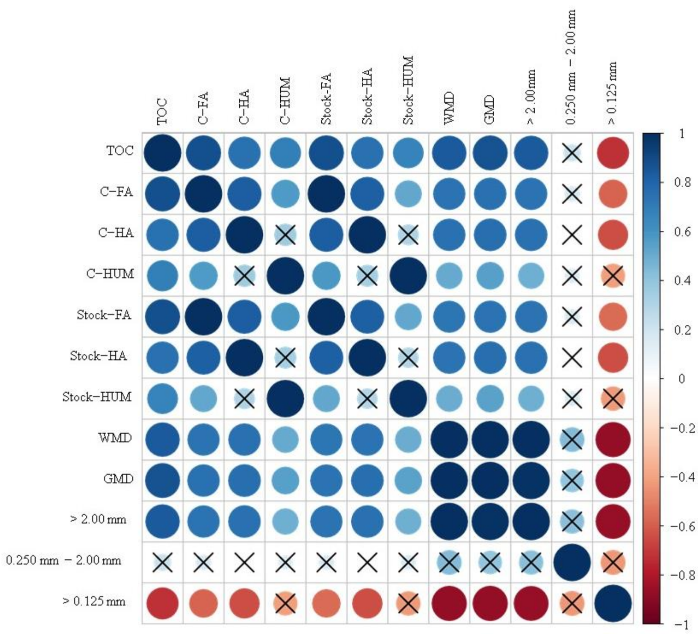

Figure 6. Pearson's correlation between the variables: total organic carbon (TOC), carbon of fulvic acids (C-FA), humic acids (C-HA), and humin (C-HUM), carbon stock of fulvic acids (Stock-FA), humic acids (Stock-HA) and humin (Stock-HUM), weighted mean diameter (WMD), geometric mean diameter (GMD), macroaggregates (> 2.00 mm), mesoaggregates (0.250 mm - 2.00 mm), and microaggregates (< 0.250 mm). Indications with (X) are non-significant correlations ($p \leq 0.05$).

4. DISCUSSION

The higher TOC levels observed in the PP area in three of the four strata studied (Table 2) can be explained by the potential that well-managed pastures have in accumulating C (Santos *et al.*, 2019; Segnini *et al.*, 2019). Well-managed pastures have a high potential to deposit plant material and consequently constantly renew soil TOC (Chatterjee *et al.*, 2018). In addition to the high production of biomass in the above-ground part, willows do not suffer from soil disturbance, associated with a great capacity to produce roots that reach very deep layers and, when they enter the decomposition process, contribute to increasing the TOC content in the subsoil (Zanini *et al.*, 2021).

This effect of pasture roots at depth can also be observed in the accumulation of TOC in the 0.20-0.40 m layer in NT+B, a *Brachiaria* system grown with maize as a second crop. This suggests that even with a short cultivation period of 4 years, the system has the potential to increase and possibly surpass the TOC content of NT over the years and approach PP. In the long term, it is possible that the NT+B area could increase the C content in the surface layers (Bieluczk *et al.*, 2020).

Of the humic fractions, HUM had the highest contents and stocks among the systems. As with the TOC contents, the PP system had the highest contents and consequently the highest stocks of HUM. Despite the low plant diversity in the PP systems, the high biomass produced, long establishment history, and non-transformation resulted in higher stocks of the most stable fractions in these medium texture soils (Locatelli *et al.*, 2022; Lavallee *et al.*, 2020; Signor *et al.*, 2018).

Compared to NT and NT+B, application of *Brachiaria* resulted in a 93% increase in stock HUM in the 0.10-0.20 m layer (Table 2). In the 0.20-0.40 m layer, the increase was 567%, corresponding to an average increase of 2.61 Mg ha^{-1} for each year after planting. These results, as well as those of the constituents, highlight the importance and influence of grasses, mainly through the action of their roots in deeper soil layers, which contribute to greater accumulation of organic matter and subsequent humification of this organic matter (Pires *et al.*, 2022; Mattei *et al.*, 2020; Oliveira *et al.*, 2019). Subtropical climatic conditions also favor slower decomposition compared to tropical climatic conditions, which contributes to the humification process of SOM (Pellegrini *et al.*, 2022; Leizeaga *et al.*, 2022). Even if *Brachiaria* has been cultivated in the area for a short time, this grass promotes the humification process of SOM, leading to stabilization of carbon in the soil.

The lower contents and stock-HUM in the subsurface layers of the NT system could be mainly related to the low diversity of plant substances present in this area, which do not diversify the organic matter and its recalcitrance (Petitjean *et al.*, 2019; Soares *et al.*, 2019). The successive cultivation of soybeans and corn on NT, a common practice in the region, leads to poorly diversified root systems with little development at depth, especially in the layer up to 0,20 m, which reduces the efficiency of the NT system in accumulating carbon at depth.

The higher stock-FA and stock-HA in the area PP in three of the four evaluated layers (Table 2) reflects the long time of establishment of this system (Silva *et al.*, 2022; Assunção *et al.*, 2019). The presence of higher stock-FA in systems where there is no turnover reflects the constant input of plant material into the soil and a gradual and slow decomposition process of the SOM, which not only favors the presence of less stable fractions, but also leads to an increase in the more stable fractions (stock-HUM) over the years (Silva *et al.*, 2022; Tadini *et al.*, 2022b; Assunção *et al.*, 2019; Pegoraro *et al.*, 2018; Rosset *et al.*, 2016).

Systems that efficiently produce high amounts of HS, especially the more stable fractions, are very important for agricultural production, as they help to maintain production capacity, improve cation retention and water storage in the soil (Firmino *et al.*, 2022; Tisdall and Oades, 1982). Based on these results, it is plausible to state that in areas with no-till associated with grasses (even with few years of implementation) and in well-managed pastures, the chemical structure of HS is richer, widely distributed in depth and, consequently, with higher contents and stable stocks of HS, which is directly related to the formation of stable aggregates that ensure the protection of the SOM (Machado *et al.*, 2020).

The aggregation indices WMD, GMD and OLev were higher in the area PP compared to the other areas in the two layers studied (Figure 2), which is directly related to the high TOC content in this area (Table 2). This relationship can be explained by the interaction of C with soil particles, which act as one of the main binders for the formation of aggregates (Tisdall and Oades, 1982; Jastrow, 1992; Six and Paustian, 2014; Six *et al.*, 2004, Six *et al.*, 1999). In addition to high TOC content, well-managed grasses also contribute to the processes of aggregate formation and stabilization of tropical soils, mainly due to the high density of their roots, by releasing organic exudates to the soil that stimulate microbial activity and soil fauna (Salton *et al.* 2014). Under these conditions, more complex structures are formed whose byproducts also contribute to the stabilization of aggregates (Loss *et al.*, 2011).

The results observed in the PP system are evident in several studies comparing PP and no-till systems (Medeiros *et al.*, 2022; Pinto *et al.*, 2022; 2021; Falcão *et al.*, 2020; Ferreira *et al.*, 2020; Medeiros *et al.*, 2020; Santos *et al.*, 2020; Wuaden *et al.*, 2020; Ozório *et al.*, 2019; Rosset *et al.*, 2019). No differences were observed in soil aggregation parameters in the NT, NT+B and NF areas, indicating the benefits that the production systems (NT, NT+B) have on the formation of soil aggregates, similar to the NF results. This is mainly due to the constant input of SOM by the cultural remains, apart from not disturbing the soil (Ferreira *et al.*, 2020; 2018a; Pinto *et al.*, 2021).

It is important to note that after four years, the incorporation of *Brachiaria* in the systems NT+B did not produce significant improvements in soil aggregation compared to the plot without *Brachiaria*. This can be attributed to the short implementation period, as the most significant effect of the application of grass was observed in deeper layers, in quantitative and qualitative variables of SOM (Table 2), possibly contributing to greater structuring at depths greater than 0.10 m.

As with the WMD, GMD, and OLev variables, the PP site had the highest proportion of water-stable macroaggregates. It is important to note that despite the differences compared to the PP area, the NT and NT+B areas had a proportion of macroaggregates greater than 37.28% in the 0-0.05 m layer. The formation of macroaggregates is important for productive systems because it is directly related to the infiltration of water into the soil (Rauber *et al.*, 2021), reduces the density and resistance to water penetration (Cavalcanti *et al.*, 2020; Castioni *et al.*, 2019), and favors gas exchange with the atmosphere (Lima *et al.*, 2021). Moreover, the formation of stable aggregates is the most important factor contributing to C storage, since the SOM is trapped in the aggregates (Vicente *et al.*, 2019; Rosset *et al.*, 2019; Oliveira *et al.*, 2019) and is protected from the attack of decomposers (Salgado *et al.*, 2019; Assunção *et al.*, 2019); on the other hand, the aggregates benefit from the C, which has cementing properties when formed (Sarto *et al.*, 2020; Batistão *et al.*, 2020).

Based on the PCA analysis (Figure 5), it was clear how the PP domain stands out from the others. The results confirm the potential that well-managed pastures have to increase quantity (Oliveira *et al.*, 2021; Martins *et al.*, 2020; Santos *et al.*, 2020; Troian *et al.*, 2020; Knicker *et al.*, 2012) and quality (Assunção *et al.*, 2019; Silva *et al.*, 2019a; Rosset *et al.*, 2022) of SOM, as well as promoting soil aggregation (Rosset *et al.*, 2019; Vicente *et al.*, 2019). The PCA results also show the grouping of the NT and NT+B areas with the NF area, indicating that these studied production areas did not induce changes in the evaluated variables compared to the NF area, a pattern already observed by other authors (Medeiros *et al.*, 2022; Ferreira *et al.*, 2020; Gmach *et al.*, 2019).

The results indicate the importance of TOC and HS in the formation of aggregates (Tisdall and Oades, 1982; Jastrow, 1992; Six and Paustian, 2014; Six *et al.*, 2004), especially due to the low clay contents in the study area (Muggler *et al.*, 1997; Castro and Logan, 1991). This relationship between macroaggregates and TOC and HS contents favors the productivity of these areas, as this relationship between SOM and aggregate stability directly affects soil properties (Moitinho *et al.*, 2020), since these aggregates protect the SOM, especially the more stable fractions (Assunção *et al.*, 2019; Merlo *et al.*, 2022; Pessoa *et al.*, 2022), thus ensuring the storage of this C in the soil (Nascimento *et al.*, 2022).

5. CONCLUSIONS

Permanent pasture with 44 years of implementation increased the content of total organic carbon and humic substances in relation to the area with native vegetation.

The system of no-till with 24 years of implementation and no-till + *Brachiaria* with 4 years of implementation of the consortium favored the carbon contents of fulvic acids, humic acids and humic fractions.

The no-till + *Brachiaria* systems with only 4 years of implementation helped increase the carbon content of the more recalcitrant and chemically stable fractions compared to the no-till systems without the use of *Brachiaria.*

The permanent grassland area provided an improvement in soil aggregation, which can provide greater soil resistance to physical degradation.

The increase in the carbon content of fulvic acids, humic acids and humic substances contributes to the formation of stable aggregates, improving the quality of medium texture agricultural soils.

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